DNA ENCAPSULATION AND RELEASE FROM WATER-DISPERSIBLE CARBON NANO-TEST-TUBES

Yasuto Hoshikawa, Yasuyuki Kanno, Hironori Orikasa, Ittisanronnachai Somlak, Takehiko Wada, Fumi Nagatsugi and Takashi Kyotani

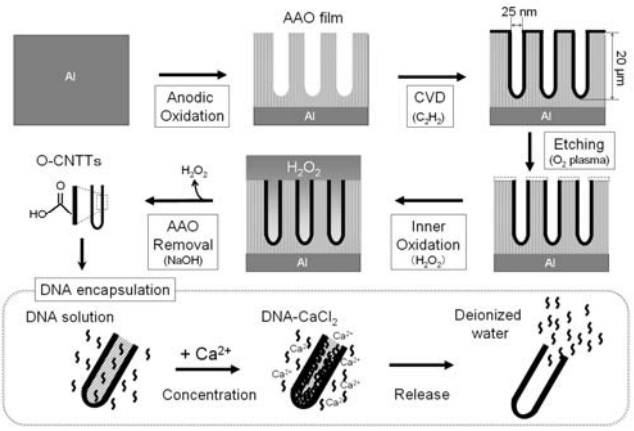
Institute of Multidisciplinary Research for Advanced Materials (IMRAM), Tohoku University, Sendai, Japan, 1-1-2 Katahira, Aobaku, 980-8577

Introduction

Tubular nano-carbons, such as carbon nanotubes (CNTs), are currently attracting significant interest as transporters for gene delivery. However, CNTs are required to overcome a major drawback of their poor dispersibility in aqueous solution for such biological applications. Water-dispersible CNTs can be obtained by the functionalization of their outer surface. Some of the CNTs carried biomolecules such as gene into cell and they showed superior endocytosis properties [1,2]. In all cases, the genes are supported on the outer surface via the bonding with the functional groups. There is, however, a limitation of the amount of genes supported on the outer surface of CNTs. More importantly, such exposed genes are easily damaged by various enzymes in biological environment during the transportation. One approach to address these issues is the use of the inner cavity of nano-carbon as storage space for genes. CNTs are, however, not suitable for such gene containers because both ends are usually closed and their inner size is too small. The template method using straight nano-channels of an anodic aluminum oxide (AAO) film is known as a useful technique to obtain uniform tubular nano-carbons with a large cavity [3]. Recently, we have synthesized carbon nano-test-tubes (CNTTs); one end of each tube is always open but another end closed. CNTTs are dispersible in water without any-post-modification [4]. Here we demonstrate the encapsulation of single-strand DNA molecules and their controlled release from the cavities of CNTTs.

Experimental

Uniformed sized CNTTs (ca. 35 nm in outer diameter, 5 nm in wall thickness and 20 μm in length) are used in this study. They were prepared by using the AAO template method, as illustrated in Scheme 1. An AAO film was uniformly carbon-coated by thermal decomposition of acetylene at 873 K and then the external carbon layer coated on the top surface of the AAO film was removed by oxygen plasma etching. To increase hydrophilicity, the inner surface of the carbon-coated nanochannels was oxidized by the impregnation with H_2O_2 solution at 323K for 4.5 h. In consequence of AAO removal by NaOH treatment, inner-oxidized CNTTs (O-CNTTs) are liberated. Note that some oxygen-containing functional groups are formed on the outer surface of CNTTs when an AAO film is dissolved with NaOH treatment, and the good dispersibility



Scheme 1 Preparation of carbon nano-test-tubes with oxidized inner walls (O-CNTTs), and the processes of DNA encapsulation and release from O-CNTTs.

can be ascribed to the formation of such groups on the outer surface [4]. The DNA used in this study is a single-strand thymine 20-mer terminated with a fluorescein molecule (its total molecular weight is 6560). O-CNTTs (0.03 mg) were put in 4 μM DNA solution (0.9 mL), with and without 3 mM $CaCl_2$, respectively. After soaking in the solution for 2 h, the sample suspension was dropped on a micro-grid and the dried CNTTs were observed with a transmission electron microscope (TEM). For comparison, non-oxidized CNTTs were prepared without the H_2O_2 treatment (hereafter referred to as Non-CNTTs) and they were also examined in the same manner as O-CNTTs.

Results and Discussion

Figure 1(a), (b) shows TEM images of O-CNTTs after the soaking in the two types of DNA solutions. Without $CaCl_2$ additive, all the tubes of O-CNTTs look empty (Fig. 1(a)). On the other hand, with $CaCl_2$ additive, there are some substances in the tube cavities (Fig. 1(b)). Such substances were also observed for Non-CNTTs when $CaCl_2$ was added, but the amount of the substances is much smaller than in O-CNTTs. The substances in O-CNTTs were subjected to elemental analysis using an energy dispersive X-ray (EDX) spectroscopy attached TEM, and it showed a peak of P (phosphorous) together with strong peaks of Ca and Cl (see the inset in Fig. 1(b)). Probably the P can be attributed to phosphate groups in the DNA. The filled O-CNTTs were separated from the DNA-$CaCl_2$ solution by centrifugation and they were dispersed in deionized water. After soaking for 2 h, the O-CNTTs were again observed with TEM. Surprisingly, as shown in Figure 1(c), almost no substance remains in the tube cavities. The supernatant water in the O-CNTTs suspension was analyzed with matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI TOF-MASS), which detected only a sharp peak at 655 (m/z) (see Fig. 1(d)). The position corresponds to the molecular weight of the DNA within instrumental error. These findings clearly indicate that O-CNTTs can encapsulate DNA in their cavities and release it into deionized water. Moreover, we can conclude that both

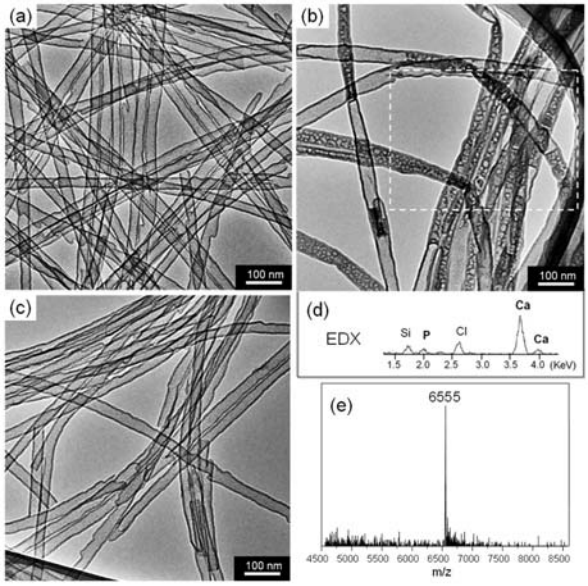


Fig. 1 TEM images of O-CNTTs after soaking DNA solution (a) without $CaCl_2$, (b) with $CaCl_2$ (DNA-$CaCl_2$) and (c) after replacing DNA-$CaCl_2$ solution with deionized water. (d) an EDX spectrum from the area indicated by a dotted square in the TEM image (b), (e) MALDI-TOF-MASS peaks from the supernatant water the suspension of the soaked O-CNTTs and deionized water.

encapsulation and release are controlled just by changing the concentration of $CaCl_2$ in the solution.

The amount of DNA released in the water, which is approximately equal to the amount of DNA encapsulated, was determined by fluorescence analysis to be 1.03×10^{-15} g per each CNTT. This value corresponds to 94,521 DNA molecules in each CNTT. If the tube cavities are simply filled with the 4 μM DNA solution, the number of DNA molecules in each CNTT is calculated to be only 24. This means that, in the DNA-$CaCl_2$ solution, DNA molecules were condensed to approximately 4,000 times in the nano-space of each CNTT cavity. Note that we confirmed that no DNA condensation occurred during the drying process for the TEM observation.

The present results suggest that the H_2O_2 treatment and the addition of $CaCl_2$ are two important key factors affecting the DNA encapsulation. The surface functional groups on both O-CNTTs and Non-CNTTs were analyzed by a temperature-programmed desorption (TPD) technique, which revealed that the amount of carboxyl group (-COOH) on the inner carbon surface in O-CNTTs (0.249 mmol/g) is three times as large as that in Non-CNTTs (0.086 mmol/g). This implies that the H_2O_2 treatment significantly improved the hydrophilicity of the inner carbon surface and the introduction of the DNA solution into the tube cavities became easy as a result.

It has been reported that a similar DNA aggregation so called “DNA compaction” occurs in the presence of metal cations in water solution [5]. However, in this study, we have never observed such DNA aggregation in the DNA-$CaCl_2$ solution, but witnessed the DNA condensation only in the cavities of CNTTs. Taking this finding into consideration, we can regard the observed condensation as a phenomenon

Table 1. The Amount of Encapsulating DNA in Each O-CNTTs and the Salt Concentration in Each DNA Solution

		NaCl	KCl	$CaCl_2$	$MgCl_2$
DNA molecules/CNTT	($\times10^{-16}$ g)	4.8	6.8	10.3	11.9
	(pcs)	44,049	62,402	94,521	109,204
Salt concentration	(mM)	9.0	9.0	3.0	3.0

peculiar to such nanospace as the CNTT cavity. In other words, our study indicates that nanospace can induce DNA condensation. For further understanding of the effect of metal cation, we added other types of salts, such as $MgCl_2$, NaCl and KCl. Note that monovalent cationic salts (Na^+ and K^+) were added three times more than bivalent ones (Ca^{2+} and Mg^{2+}) to keep the same ionic strength. The amount of DNA encapsulated into O-CNTTs with the addition of each salt is summarized in Table 1. The amount differs in the type of salt depending on the cation valence; bivalent ions as Ca^{2+} and Mg^{2+} show more encapsulation than monovalent ones as Na^+ and K^+. Since we keep ionic strength constant, the difference in the amount (Table 1) can’t be solely explained by the salting-out. Thus the DNA condensation is not only controlled by electrostatic force. Probably bivalent metal cations play some role of binder between a base in a DNA molecule and a phosphate group in another molecule, as suggested in the literature [5]. Our study implies that such binder effect can be enhanced in nanospace.

Conclusions

We found that, with the addition of $CaCl_2$ to the DNA solution, the DNA molecules were introduced into the cavities of CNTTs as long as their inner surface is hydrophilic. Moreover, the DNA was released from CNTTs just by decreasing the concentration of $CaCl_2$. These features clearly demonstrate promise for the use of CNTTs as gene carriers. Moreover, we discovered that DNA condensation is induced by the nanospace in the CNTT cavities. This phenomenon may attract a great interest from many researches who investigating DNA compaction and chromosome packing, and may be useful to develop a novel technique for DNA purification and separation system using nanospace.

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